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TRANSFER WITH DIALYSABLE TRANSFER FACTOR OF T-LYMPHOCYTE CYTOLYTIC RESPONSE TO TICK-BORNE ENCEPHALITIS VIRUS ANTIGEN IN NAIVE MICE

V. MAYER, E. GAJDOŠOVÁ, *O. ORAVEC

Institute of Virology, Slovak Academy of Sciences, 809 39 Bratislava; and *Institute of Experimental Oncology, Slovak Academy of Sciences, 881 32 Bratislava, Czechoslovakia

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Summary. — Transfer factor activity was demonstrated in the dialysable extract from lymphocytes from outbred donor mice, in which immunization with live attenuated Langat (E5 "14") virus induced a state of high resistance against challenge with virulent tick-borne encephalitis virus. Administered to naive inbred recipient mice, the extract coneyevd in them specific cytolytic activity, exerted by their T lymphocytes, as demonstrated by the ^{51}Cr release assay on tick-borne encephalitis virus-infected syngeneic L-929 cells.

Key words: tick-borne encephalitis virus; transfer factor; cytolytic T lymphocytes; ^{51}Cr release assay

Numerous investigations following the initial discovery by Lawrence (1955) of the "transfer factor" (TF) have confirmed that dialysable extracts from human blood leukocytes isolated from immune donors have the capacity to transfer delayed-type cutaneous hypersensitivity (DCH) to non-immune recipients. The term TF signifies rather a specific functional entity contained in the raw dialysate, possibly of a polypeptidic-ribonucleotidic nature with a molecular weight of 10,000 (Kirkpatrick and Smith, 1977; Rifkind *et al.*, 1977). TF, a product of immunocompetent cells, seems to convert in the recipient the lymphoid cells of thymus-dependent lineage in a state of antigen responsiveness. TF is recognized as one of the most potent immunological reagents to initiate, restore and amplify the cell-mediated component of the immune response (CMI) (Lawrence, 1974), proven e.g. in the treatment of severe fungal infections in immunocompromised individuals (e.g., Graybill, 1974).

Accumulated data suggest that TF preparations exert a) apparent immunological specificity, i.e. cause de novo appearance of new clones of antigen-reactive T lymphocytes, essential for generation of DCH in the recipient (antigen-dependent effects), and b) biological activities in the absence of the antigen, possibly important in modulation of the CMI response (Kirkpatrick and Smith, 1977).

Little attention has so far been paid to the study of CMI phenomena transferable by TF in experimental viral infections, especially in relation to antiviral defence elicited by immunization with attenuated viruses. The mechanisms underlying the highly protective and long-standing effects of live viral vaccines — at least against viruses causing systemic infections — are unclear, although an important role seems to be attributable to CMI, as suggested by experiments with viruses of the tick-borne encephalitis (TBE) complex, exhibiting different degrees of mouse virulence (Mayer *et al.*, 1976; Mayer and Mitrová, 1977; Gajdošová and Mayer, 1978; Gajdošová *et al.*, 1980). To assess the role of CMI in the marked resistance (against virulent challenge) conferred in mice by the antigenically close attenuated Langat E5 "14" virus (Mayer, 1975), we investigated the generation and the effect of TF in this system. Instead of the routine DCH tests we used the quantitative ^{51}Cr release assay measuring, moreover, the response only of one specific subpopulation of thymus-dependent lymphocytes, the cytolytic killer cells.

We are reporting the first basic observations on the in vivo transfer of cellular cytotoxicity directed against TBE virus (western subtype)-infected cells by dialysable TF prepared from splenic lymphocytes of mice given the attenuated Langat (E5 "14") virus.

Subadult SPF, outbred donor mice ("ICR" strain) were given subcutaneously (s.c.) one dose of 10^3 PFU of Langat virus reproduced in SPF chick embryos. Another group of mice, matched controls, received 0.14 M NaCl solution. After 19 days the animals were killed and the dialysable TF was extracted from spleen cells according to Lawrence (1955) for use with human peripheral leukocytes, as modified by Rifkind *et al.* (1977) for the murine system. Splenocytes from mice of the experimental and control groups were harvested separately from their mechanically coarsely minced spleens. After thorough washings in Eagle's basal medium they were resuspended to a concentration of 10 cells/ml in bidistilled water. $MgSO_4$ (50 mg per ml of suspended cells) and a few crystals of pancreatic deoxyribonuclease (SERVA) were added and the cells of either group were subjected to 10 cycles of freezing and thawing in a dry ice-alcohol bath. The resulting cell lysate was dialysed twice against 25 volumes of bidistilled water for 18 hr at 4 °C. The respective dialysates were pooled and concentrated by freeze-drying. The residual substance, originating from splenocytes either from immunized or from NaCl solution-injected mice was dissolved in saline so that each ml of TF or placebo represented its amounts extracted from three spleens. These materials were stored in portions at −78 °C until used. TF material contained no infectious virus.

To test the TF activity, naive subadult male inbred C3H mice were used as recipients. Cohort mice, divided into groups of 8 animals, were given TF either intraperitoneally (i.p.) or intravenously (i.v.). Two or three days after discontinuation of the TF dosage, recipient mice were killed, their spleens removed and pooled isolated cells used for the cytolytic assay performed in triplicate.The group "E" of recipient mice received the extract from splenocytes of non-immunized mice. The cytolytic activity of lymphocytes was measured on TBE virus (western subtype)-infected L-929 cell monolayers, labelled with 0.18 MBq of ^{51}Cr ($Na\,^{51}CrO_4$ with a specific activity of 3.7 — 14.8 MBq per mg of chromium) at an effector: target cell ratio of 100:1, as described (Gajdošová *et al.*, 1980). C3H mice and L-929 cells represent syngeneic system, sharing the K locus of the H-2 gene complex, specifying the major histocompatibility system in the mouse.

Lymphocytes from the mice of groups A-D and the E group were incubated overnight with ^{51}Cr -labelled uninfected or TBE virus-infected L cells. Several important features were observed (Table 1). TF, injected by either route, induced in the non-immune recipient mice a significant cytolytic reactivity, directed allegedly against the virus-infected target cells, but without a demonstrable effect on the uninfected cells alone. Calculated by Student's t-test,

Table 1. Cytolytic activity of spleen effector cells from naive C3H mice administered the Langat TP-21 transfer factor [1]), demonstrated on TBE virus-infected target L-929 cells

Groups of 8 recipient mice	Treatment: Dosage, route, timing	Cytolytic activity measured on the indicated day after the last dose	^{51}Cr release (%) from target cells[2]) uninfected	infected	P
A	Langat transfer factor 2 doses, i.p., on days 0, +1	3	23.68±1.27	42.14±4.57	<0.01
B	3 doses, i.p., on days 0, +1, +2	2	24.44±1.31	46.27±4.38	<0.005
C	2 doses, i.v., on days 0, +1	3	24.70±1.07	53.44±2.80	<0.001
D	3 doses, i.v., on days 0, +1, +2 Dialysable material from normal spleen leukocytes[3])	2	23.37±2.16	57.30±4.01	<0.001
E	2 doses, i.p., on days 0, +1	3	22.11±0.70	18.69±0.56	<0.5
Spontaneous	^{51}Cr release from labelled L cells		16.67±0.70	21.17±0.43	<0.5

[1]) Dialysable leukocyte extract from spleens of subadult outbred mice immunized with one s.c. dose of 10^3 PFU (0.1 ml of the live attenuated Langat TP-21 E5"14" virus.

[2]) Results are expressed as the mean percentage of ^{51}Cr release ± standard errors of the mean from 3 measurements. L-929 cells were infected at an input multiplicity of 2 PFU of the "204" strain of TBE virus (western subtype) per cell.

[3]) Dialysable leukocyte extract from spleens of subadult outbred mice given 0.1 ml of a 0.14 M NaCl solution i.v.

Dosage: 0.25 ml i.p., 0.1 ml i.v.

the differences in ^{51}Cr release between these two target cell groups were highly significant, when lymphocytes from TF-treated mice were assayed. No significant differences in ^{51}Cr release, however, were observed between the TBE-infected and uninfected L cells with lymphocytes from placebo-injected recipient mice. Similarly, no significant differences were noted in spontaneous ^{51}Cr release values between infected and uninfected L cells in the absence of any lymphocytes.

TF was significantly more active when administered i.v. than i.p., though a 2.5 times lower amount of TF was administered by the former route. If comparing group A with C, P was 0.025, if the group B with D, P was 0.05. Because human TF is usually injected intradermally or s.c., the i.v. route appears worth of considering in the treatment of more severe cases.

Dialysates of spleen lysates from outbred donor mice, rendered resistant against virulent TBE virus challenge by single-shot active immunization with a relatively low dose of the attenuated Langat virus, were shown to convey the cytolytic reactivity — one of the important CMI responses — against the TBE virus neoantigens, conceivably present on the infected targe cell surface. This cytolytic reactivity, as shown in previous experiments, on the same H-2K syngeneic system, is exerted by the T cells (Gajdošová *et al.*, 1980).

Sensitized, thymus-dependent cytolytic lymphocytes recognize the viral antigen only under conditions of restriction by the major histocompatibility locus, discriminating clearly the "self" of virus-infected cells. On the other hand, it is firmly established that the presense of virus alone or the respective H-2 locus does not elicit in mice effector : target cell interactions, measurable by available techniques (Doherty *et al.*, 1978). The current idea concerning the generation of virus-specific CMI response assumes in the host an initial interaction between the precommited, virgin T lymphocyte and the virus-infected stimulator cell, which may be a macrophage (Dunlop and Blanden, 1976) or possibly other specialized cell populations (Nakshima and Lake, 1979), "presenting" the antigen. The stimulator cell and the target cell must share the genes mapping — in our case — the H-2K locus. This identity is not required between T effector cell and target cell. The stimulatory cell's signals for the "self" and "non-self" (i.e. viral neoantigen) induce in the virgin T lymphocyte the ability to recognize both these structures on the target cell in order to function as cytotoxic killer. Because our TF was prepared from apparently allogeneic splenocytes originating from outbred mice, it is tempting to hypothetize that TF, for antigen-dependent effect studied, may act at the level of stimulatory cells. Obviously, to prove that such an idea is correct, the nature of basic interactions at the level of stimulatory function a) between the "self" and "non-self" genes and b) between them and the still unclear mechanisms of (purified) TF action, need to be elucidated. In spite of the complexity of the problem it is encouraging to note that TF, being actually a "passive" form of "active immunization" may participate in the state of long-lasting resistance conferred by live virus vaccines and even in augmentation of immune response (Mayer *et al.*, in preparation). Similarly, the possibility that TF may broaden the modest contemporary list of antiviral factors should not be overlooked.

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